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GENERATION OF HIGHLY DIVERSE LIBRARY OF EXPRESSION VECTORS VIA HOMOLOGOUS RECOMBINATION IN YEAST

Inventors: Li Zhu and Shaobing Hua

ABSTRACT

Methods are provided for generating highly diverse libraries of expression vectors encoding fusion proteins such as single-chain antibodies via homologous recombination in yeast. The method comprises: transforming into yeast cells a linearized yeast expression vector having a 5'- and 3'-terminus sequence at the site of linearization and a library of insert nucleotide sequences that are linear and double-stranded; and having homologous recombination occur between the vector and the insert sequence such that the insert sequence is included in the vector in the transformed yeast cells. The insert sequence comprises a first nucleotide sequence encoding a first polypeptide subunit, a second nucleotide sequence encoding a second polypeptide subunit, a linker sequence encoding a linker peptide that links the first and second polypeptide subunits, and a 5'- and 3'- flanking sequence at the ends of the insert sequence which are sufficiently homologous to the 5'- and 3'-terminus sequences of the linearized yeast expression vector, respectively, to enable homologous recombination to occur. The first polypeptide subunit, the second polypeptide subunit, and the linker polypeptide are expressed as a single fusion protein; and the first and second nucleotide sequences each independently varies within the library of expression vectors.